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# Changes in the Epidemiology of Hepatitis C Infection in Germany: Shift in the Predominance of Hepatitis C Subtypes

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Hepatitis C virus (HCV) subtype distribution was studied in 395 chronically infected patients from Germany. HCV genotype 1 was most frequent (80.5%). One hundred forty-three individuals (36.2%) were infected with subtype 1a and 175 (44.3%) were suffering from subtype 1b infection, respectively. HCV subtype 3a was found in 53 (13.42%) persons. Subtypes 2a, 2b, and 2c have been detected in 5 (1.27%), 10 (2.53%), and 4 (1.01%) individuals. Genotypes 4 and 5a accounted for HCV infections in 4 (1.01%) and 1 (0.25%) subjects. There was a notable variation in the distribution of the prevalent subtypes 1a and 1b in different age groups. Subtype 1a was detected in 53.3% and 68.0% of patients aged 1–10 and 11–20 years, whereas subtype 1b in the same groups was present only in 33.3% and 28.0% of patients, respectively. In contrast, in individuals older than 50 years subtype 1b was most frequent. Thus, subtype 1b has been gradually substituted for subtype 1a during the last 20 years. Logistic regression analysis with adjustment for sex and different modes of HCV acquisition demonstrated that age of the infected subjects was a direct explanatory variable for subtype 1a and 1b distribution. Therefore, the observed shift in HCV subtype prevalence could not be attributed to changes in the epidemiological relevance of different known risk factors of HCV transmission, as had been assumed in previous studies. The altered subtype pattern reported here may have a profound influence on the future epidemiology of HCV infection. ***J. Med. Virol. 60:122–125, 2000.***



**KEY WORDS:** hepatitis C virus; genotypes; age distribution; epidemiology

## INTRODUCTION

Recently, a tendency for a change in the prevalence of the predominant hepatitis C virus (HCV) types was noted in several European countries and the United States [Nousbaum et al., 1995; Silini and Mondelli, 1995; Bell et al., 1996; Zein et al., 1996; Berg et al., 1997; Feucht et al., 1997; Viazov et al., 1997]. The studies conducted, however, have not been conclusive, mainly due to the absence of information on HCV genotype prevalence in younger age groups and the lack of comprehensive statistical analysis. Considering that the shift in HCV genotype distribution could have a significant impact on practical medical issues (e.g., changes in the spectra of clinical HCV manifestation or in the efficacy of interferon treatment [Silini and Mondelli, 1995]), we addressed these unresolved questions by studying the HCV subtype distribution in a group of 395 German patients with chronic HCV infection, including 65 HCV-infected individuals younger than 20 years.

## PATIENTS AND METHODS

Sera from 395 (241 men, 154 women) chronically HCV infected in- and outpatients attending Essen University Hospital between 1994 and 1997 were included in this study. The age, sex, and the source of HCV infection of the individuals enrolled is shown in Table I. All patients were positive for HCV antibodies by a second generation immunoassay (Abbott Diagnostics) and for HCV RNA by diagnostic reverse transcription-polymerase chain reaction (RT-PCR; Monitor, Roche Diagnostics). One hundred three (26.1%) patients had a history of transfusion with blood products, 78 (19.7%) were intravenous drug users (IVDU), and 29 (7.3%) underwent hemodialysis. No particular parenteral risk factor could be identified with certainty for the remaining patients. It is highly likely, however, that a significant number of them experienced blood transfusions or other parenteral exposures in the past.

Viral RNA was reverse transcribed with primers de-

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TABLE I. Age, Sex Distribution, and Source of HCV Acquisition in Chronically Infected Patients*

| Age (years) | N | | | Source of HCV acquisition (N) | | | |
|---|---|---|---|---|---|---|---|
| | Total | Males | Females | Dialysis | Transfusion | IVDU | Unknown |
| 1–10 | 15 | 8 | 7 | — | 8 | — | 7[a] |
| 11–20 | 50 | 25 | 25 | 1 | 39 | 3 | 7 |
| 21–30 | 58 | 44 | 14 | — | 14 | 20 | 24 |
| 31–40 | 94 | 64 | 30 | 9 | 10 | 42 | 33 |
| 41–50 | 71 | 46 | 25 | 6 | 11 | 11 | 43 |
| 51–60 | 56 | 22 | 34 | 9 | 5 | 2 | 40 |
| 61–70 | 37 | 23 | 14 | 4 | 12 | — | 21 |
| >70 | 14 | 9 | 5 | — | 4 | — | 10 |

*HCV, hepatitis C virus; IVDU, intravenous drug use.
[a]Perinatal HCV transmission probably occurred in four of these individuals.

rived from the core region of the HCV genome: p874A: 5′-A[A, G]GAAGATAGA[A, G]AA[A, G]GAGCAACC-3′ (RT and PCR 1), p417S: 5′-GG[C; T]GG[C, T]GG[A, G, C, T]CAGATCGTTGG-3′ (PCR 1), p439S: 5′-GAGT[A, T]TAC[G, T, C]TG[C, T]TGCCGCGCAG-3′ (PCR 2), and p1AS: 5′-AT[A, G]TACCCCATGAG[A, G]TCGGC-3′ (PCR 2) [Viazov et al., 1997]. The DNA fragments obtained were typed by DNA enzyme immunoassay (DEIA, Sorin), as described in detail elsewhere [Viazov et al., 1994]. For HCV genotyping, the nomenclature of Simmonds et al. [1996] was used. Twelve HCV isolates not typeable by DEIA were sequenced directly in both directions using a Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). The obtained nucleotide sequences were subjected to phylogenetic analysis with the package PHYLIP, version 3.5 [Felsenstein, 1993]. Types were determined by comparison with HCV core sequences drawn from the GenBank and were shown to be subtypes 1a (3 isolates), 1b (1 isolate), 2a (2 isolates), 2b (4 isolates), 4 (1 isolate), and 5a (1 isolate).

### Statistical Analysis

All statistical analyses were performed with statistical software SAS. The dependence of HCV genotype 1a and 1b prevalence on the explanatory variables age, sex, and modes of HCV acquisition was analysed by logistic regression analysis [Cox and Snell, 1989]. Differences regarding modes of HCV acquisition were assessed using 95% confidence intervals (CI) that correspond to exact confidence limits of binominal distribution [Ciba-Geigy, 1980; Armitage and Berry, 1994].

## RESULTS

In the 395 patients studied, HCV genotype 1 was most frequent (80.5%). One hundred forty-three individuals (36.2%) were infected with subtype 1a and 175 (44.3%) were suffering from subtype 1b infection, respectively. HCV subtype 3a was found in 53 (13.42%) persons. Subtypes 2a, 2b, and 2c could be detected in 5 (1.27%), 10 (2.53%), and 4 (1.01%) individuals. Genotypes 4 and 5a accounted for HCV infections in 4 (1.01%) and 1 (0.25%) subjects. No coinfection with another genotype or subtype could be observed in any case.

Figure 1 demonstrates a notable variation in the distribution of the prevalent subtypes 1a and 1b in different age groups. Subtype 1a was detected in 53.3% and 68.0% of patients aged 1–10 and 11–20 years, while subtype 1b in the same groups was present only in 33.3% and 28.0%, respectively. In individuals 21–30 years old, subtype 1a was also statistically most prevalent (53.5%). In the next two groups (31–40 and 41–50 years old), the numbers of individuals infected with HCV subtypes 1a and 1b were comparable (32–41%). In patients older than 50 years, subtype 1b was statistically predominant, accounting for almost 80% of all infections observed. Subtype 3a was detected mainly in young and mature adults (21–50 years). The single cases of infections with other HCV types occurred in individuals of different age without any regularity. Regarding the source of HCV infection (Table II), subtype 1a was most frequent in patients with a history of blood transfusions and in IVDU. Patients on hemodialysis and those who contracted HCV infection by unknown modes were mostly infected by HCV genotype 1b. These two groups, however, mostly consisted of individuals older than 30 years. Additionally, it is highly probable that a significant number of those without evident modes of HCV acquisition experienced blood transfusions or other parenteral exposures in the past, but were not aware of such events.

In general, our results demonstrated a predominance of HCV subtype 1a in younger age groups and of HCV subtype 1b in older individuals. This observation might reflect a tendency for a gradual change in the spectrum of HCV variants circulating in the German population. An alternative explanation could be that the ratios of patients infected with HCV by different routes in each age group are unequal. To differentiate between these two possibilities, logistic regression analysis was performed. After adjustment for sex and modes of HCV transmission, age of the infected individuals proved to be a direct explanatory variable for HCV subtype 1a and 1b distribution ($P = 0.0001$). The odds ratio calculated was 0.62 (confidence interval [CI] = 0.53–0.73), indicating that the probability of type 1a

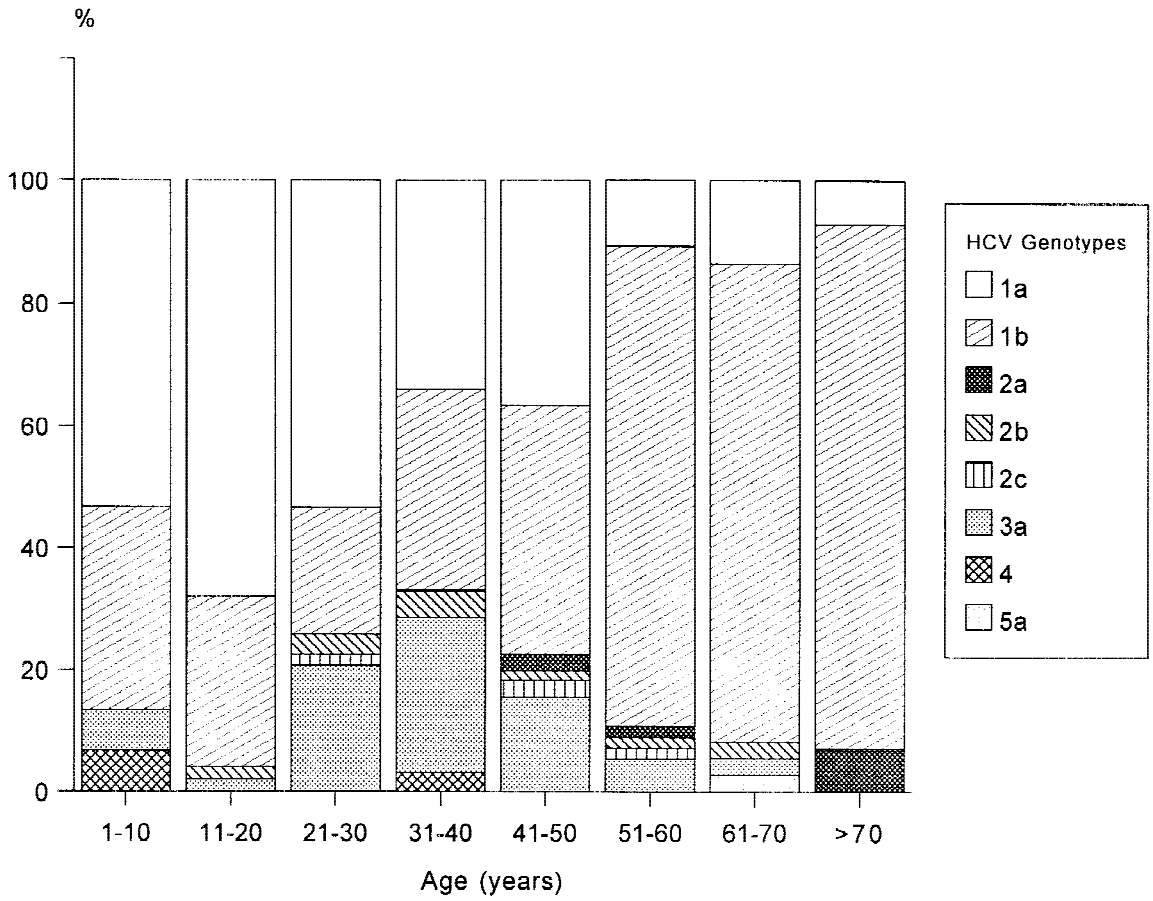


Fig. 1. Distribution of hepatitis C virus (HCV) subtypes in different age groups.

TABLE II. HCV Subtype Distribution According to the Modes of HCV Acquisition*

| HCV subtype | Modes of HCV acquisition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dialysis | | | Transfusion | | | IVDU | | | Unknown | | |
| | *N* | % | 95% CI[a] | *N* | % | 95% CI | *N* | % | 95% CI | *N* | % | 95% CI |
| 1a | 3 | 10.3 | 2.19–27.35 | 53 | 51.5 | 40.52–60.26 | 40 | 51.3 | 39.68–62.77 | 47 | 25.4 | 19.89–39.18 |
| 1b | 20 | 69.0 | 49.57–84.72 | 44 | 42.7 | 32.45–51.96 | 9 | 11.5 | 5.41–20.78 | 102 | 55.1 | 47.97–62.89 |
| 2a | — | — | — | 1 | 1.0 | 0.02–5.19 | 1 | 1.2 | 0.02–6.94 | 3 | 1.6 | 0.35–4.79 |
| 2b | 2 | 6.9 | 0.85–22.77 | 2 | 1.9 | 0.23–6.71 | 2 | 2.6 | 0.31–8.96 | 4 | 2.2 | 0.61–5.59 |
| 2c | 1 | 3.45 | 0.09–17.26 | 1 | 1.0 | 0.02–5.19 | — | — | — | 2 | 1.1 | 0.14–3.96 |
| 3a | 2 | 6.9 | 0.85–22.77 | 2 | 1.9 | 0.23–6.71 | 24 | 30.8 | 20.81–42.42 | 25 | 13.5 | 9.20–19.84 |
| 4 | 1 | 3.45 | 0.09–17.26 | — | — | — | 2 | 2.6 | 0.31–8.96 | 1 | 0.55 | 0.01–3.06 |
| 5a | — | — | — | — | — | — | — | — | — | 1 | 0.55 | 0.01–3.06 |

*HCV, hepatitis C virus; IVDU, intravenous drug use; CI, confidence interval.
[a]95% confidence intervals that corresponds to exact confidence limits of binominal distribution.

infection in a given age group is only 0.62 times the risk encountered in the preceding one.

## DISCUSSION

In the current study, HCV genotypes were determined in 395 chronically HCV infected patients. Two important characteristics of this study should be noted. First, because there was no selection, our patient cohort can be regarded as a representative sample of all individuals with clinically apparent HCV infection, at least from one German area. Second, inclusion of a considerable number of HCV infected children and young adults allowed for a more precise determination of the peculiarities of HCV subtype distribution according to age. We showed that HCV subtype 1b was most prevalent in chronically infected individuals older than 50 years. On the other hand, subtype 1a accounted for 53.3% and 68.0% of all HCV infections observed in patients younger than 21 years. Our findings are complemented by several recent studies, which indicated an

increase in the number of HCV 1a infections in the adult population of France, Italy, Germany, Norway, the former Soviet Union, and the United States [Nousbaum et al., 1995; Silini and Mondelli, 1995; Bell et al., 1996; Zein et al., 1996; Feucht et al., 1997; Viazov et al., 1997]. Taken together, these data provide direct evidence for a shift from HCV subtype 1b to subtype 1a during the last 20 years in Germany and most probably in some other countries.

The reasons for the observed shift remain unknown. In general, HCV subtype distribution might be influenced by a variety of factors, in particular by changes in the epidemiology of HCV infection. One might suggest that the shift in HCV subtype prevalence is attributable to a change in the relative importance of different known risk factors of HCV transmission, facilitating the spread of particular HCV subtypes. Thus, for example, it was shown that subtype 1a could have been transmitted more frequently than subtype 1b by blood transfusions during recent years [Pol et al., 1995]. Initially, the high prevalence of subtype 1a in young HCV infected patients with a history of blood transfusion seems to be in favor of this notion. Logistic regression analysis with adjustment for sex and different modes of HCV acquisition, however, did not support this assumption and demonstrated clearly that for our patient cohort the age of the infected individuals is a direct explanatory variable for subtype 1a and 1b distribution. Therefore, the shift in HCV subtype prevalence observed during the last 20 years is not caused by changes in the epidemiological relevance of different known risk factors of HCV transmission, as has been assumed previously [Berg et al., 1997; Feucht et al., 1997].

In the current study, possible effects of survivorship on age-specific HCV subtype distribution have not been assessed. Thus, it cannot be entirely ruled out that if patients with HCV subtypes other than 1b died sooner, there would be a greater representation of type 1b in older age groups. This assumption seems not likely, because HCV type 1 usually causes more severe liver disease and a faster disease progression than other HCV genotypes [Silini and Mondelli, 1995]. Alternative, although speculative, explanations of the changes in HCV subtype distribution might include different biological potentials of HCV subtypes or as yet unrecognized modes of HCV transmission.

Another important peculiarity of the described genotype distribution is the high prevalence of HCV subtype 1a (51.3%) in IVDU. This observation is in contrast to the results of studies performed several years ago [Pawlotsky et al., 1995; Stark et al., 1995; Shev et al., 1995; Roggendorf et al., 1996], which have indicated the predominance of subtype 3a among drug users. Because the number of posttransfusion infections in developed countries has already dramatically decreased after the implementation of a mandatory HCV screening of blood and blood products, intravenous drug abuse will most likely account for the vast majority of all new HCV infections in the future. Undoubtedly, that would lead to an even more pronounced replacement of HCV subtype 1b by subtype 1a in the general population.

Future epidemiological and clinical studies have to demonstrate the impact of the observed shift in HCV subtype distribution on medical practice. The changing HCV subtype pattern, however, should be considered today in the development of new diagnostic procedures and therapeutic strategies for treatment of HCV infection.

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